Efficacy comparison of T-705 and T-1106 Treatment for Dengue Virus in Rodents

MIC 401 Research Proposal
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Abstract

Dengue fever is now endemic in over 100 countries and over 2.5 billion people are at risk for infection each year. Due to the adaptive nature of the mosquito vectors, the number of geographically affected areas continues to rise at an accelerated rate. While most of the infected survive the conventionally high fever associated with Dengue, some progress into the severely critical period marked by hemorrhage and hypovolemic shock. If intense clinical care is not taken, this form of dengue can be fatal. Five known serotypes exist for dengue, but cross-reactive antibodies that cause antibody-dependent viral enhancement in secondary infection provide unique challenges in vaccine development. Recent studies have shown promising results against viruses belonging to the same family as dengue, with the use of pyrazine carboximide antivirals T-705 and T-1106 that serve as inhibitors of viral RNA-dependent RNA polymerase in rodent models. It is with consideration of these results that an efficacy comparison of T-705 and T-1106 treatment for Dengue virus in a rodent model it proposed.

Introduction

Dengue Virus (DENV) is a +sense single stranded RNA virus and a member of the *Flaviviridae* family of viruses that also include West Nile virus (WNV) and yellow fever virus (YFV). Dengue virus has 4 known serotypes (DENV-1, DENV-2, DENV-3, and DENV-4), with a 5th serotype (DENV-5) purportedly discovered in 2013 [3, 7, 8]. Transmission of DENV to humans and non-human primates occurs through its intermediate vector, mosquitoes *Aedes aegypti* and *Aedes albopictus*, which can be found throughout the world [1, 2]. Dengue virus is considered a significant threat to public health on a global scale with an estimated 390 million infections per year and more than 500,000 cases resulting in severe, potentially fatal forms of the disease [1, 3, 6]. Contraction of the virus is known to cause dengue fever (DF) as well as the more severe manifestations of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Although dengue is an old disease, with some literature suggesting outbreaks dating back to 900 AD, prior to 1970 only 9 countries had been known to have severe epidemics. Incidence of infection has not only been rising rapidly in recent years, but unprecedented outbreaks are occurring in entirely new areas; Dengue is now endemic in more than 100 countries [4, 9, 19].

The scope this research proposal focuses on potential post-infection treatment of dengue by comparative exploration of antiviral drugs that have demonstrated improved immunological activity in treating other RNA viruses in rodent models [12, 13, 14, 15, 16, 17]. The goal of the experiment is to show similarly improved immunological activity in dengue-infected rodents, serving as a preliminary tool for potential future human treatments. The antiviral treatments work by inhibiting RNA-dependent RNA polymerase, an essential protein encoded by all RNA viruses that enable viral replication.

Transmission & Characteristics

DENV propagates between humans and mosquitoes in a circular pattern of transmission. The cycle begins when the female mosquito feeds on a DENV infected human host, then subsequently on a healthy human [10]. When the mosquito first becomes infected, the virus incubates for a period of 4 to 10 days, growing in the mosquito gut, and later migrating to the salivary glands. Once a mosquito becomes infected it is capable of transmitting the virus for the remainder of its life . Humans serve as the primary carrier and multipliers of the virus, providing an easily accessible reservoir for mosquito transmission [4].

While some remain asymptomatic, DENV infection most often results in dengue fever. Symptoms of infection proceed in 3 phases: fever phase, critical period (DHF/DSS development), and recovery phase. The fever phase is characterized by high fever (104 ^{o}F), nausea, vomiting, malaise, myalgia, retro-orbital headache, and arthralgia, and typically lasts for 3-10 days. Most patients pass from the fever phase into the recovery phase, but if severe drop in platelet count is detected, patients are often monitored for DHF development. The onset of the critical period is observed between 3-7 days after initial symptoms, lasting for 24-48 hours, and is initially characterized by reduced fever (100^{o}F). The distinction of DHF/DSS is capillary leakage coinciding with cytokine storm, thrombocytopenia, and intestinal hemorrhage. If increased fluid leakage is not countered with appropriate fluid intake, DSS hypovolemic shock will occur and the patient must be monitored for mucosal, skin, and rectal bleeds. Careful monitoring is imperative for survival and procession to the recovery phase. Recovery phase is 2-4 days following the critical period and patients' improvement is immediately apparent. Notably, if patients

were inundated with excess fluid replacement during the preceding critical period, heart failure or pulmonary edema can manifest during the recovery phase, thus too much fluid loss or replacement during the critical phase are equally dangerous and can lead to death [4, 14, 21].

Immunization & Treatment

A culmination of several factors enhances the public threat posed by dengue. *A. aegypti* is a daytime feeder most active at dawn and dusk, capable of consuming multiple blood meals from a number of hosts at each feeding, while *A. albopictus* is known to be highly adaptive. Its ability to tolerate freezing temperatures and adapt to various environments has enabled its accelerated spread throughout the world. Although prevention measures, such as limiting standing water near urban areas, the use personal protection such as clothing coverings, screens, and insecticide treatments etc., are helpful and recommended, prevailing socioeconomic factors are often inhibitory to appropriate Dengue prevention and control measures. Presently, there are no vaccines for DENV, although research is ongoing [4, 21].

Pathogenicity Challenges

DENV vaccine developers face unique challenges with Dengue requiring a vaccine that must immunize against all known serotypes [14]. While infection with one serotype, can provides long term immunity to that serotype, cross-reactive antibodies generated in the initial infection serve as a means of enhancement in subsequent infection by a different serotype [19].

During the initial infection, with for example DENV-1 serotype, DENV-1 antibodies are produced, completely binding the viral envelope and preventing viral binding of the cognate receptor. The antibodies mediate endocytosis into the cell through Fc-receptors, initiating lysosomic response that prevents envelope conformational change required for endosomal breach and subsequent release of the RNA genome into the cytoplasm, allowing it to be effectively neutralized. During a secondary infection by another serotype, for example DENV-2, B cell immunological memory DENV-1 antibodies are dispatched because of perceived virion similarity, but only weakly bind the DENV-2 envelope, leaving it unneutralized. The DENV-2 virion is then able not only to bind it's cognate receptor for cell entry, but the sparsely bound antibodies mediate an additional means of cell entry through the Fc-receptor. Albeit, because the antibodies are only sparsely bound to the viral envelope, the envelope is unrestrained from executing its conformational change and is then able to release its genome into an unsuspecting, unprepared compartment of the cell. This phenomenon is known as antibody-dependent enhancement (ADE) and is directly associated with severe dengue manifestations such as DHF and DSS [5, 20].

In addition to ADE, anomalous T cell response and viral virulence factors also contribute to severity of DENV pathogenesis. In secondary infection with disparate serotypes, cross-reactive T Cells have been found to release an excess of pro-inflammatory cytokines resulting in the endothelial dysfunction that produces vascular leakage along with abnormal apoptosis activity and of inefficient anti-DENV specific response [21]. DENV is a competent virus that has evolved an array of virulence factors allowing it to simultaneously evade host immune response and expand viral replication. While vaccines

are the principle aegis against viral infections, the acute degree of pathogenicity possessed by DENV supports the imperative for antiviral research and development.

Research Objective & Activity in Other Viral Models

The *Flaviviridae* family, that includes over 70 viruses, consists of single stranded, positive sense, enveloped, RNA genomes primarily spread though arthropod vectors. yellow fever (YFV), West Nile (WNV), and Japanese encephalitis (JEV) viruses are members of this family and along with DENV, are primarily spread through mosquito vectors [18, 22, 23]. Widespread morbidity and potential for viral hemorrhagic fevers have encouraged great research efforts on how best to inhibit their pathogenicity through antivirals.

In one such study conducted by the Institute for Antiviral Research at Utah State University, a pyrazine carboximide, dubbed T-705, was found to provide mortality protection, reduced viral load and reduced viral protein expression in rodents challenged with subcutaneous WNV administration [13]. Prior studies had shown effective antiviral activity of T-705 against Influenza strains A, B and C, as well as against viruses belonging to the bunyaviridae and arenavirus families, respectively [12,15,16]. The mechanism of action for T-705 was explored against influenza by Furuta et al., and found to inhibit influenza's RNA-dependent RNA polymerase (Rdrp) function [12]. While the mechanism of action in *Morrey, Futura et al.*'s WNV T-705 study was not conclusively confirmed [17], the broad-spectrum efficacy of T-705 and mechanism identified in the *Futura et. al* study makes it an eligible candidate for DENV treatment exploration [12, 16, 17].

Another pyrazine carboxamide, T-1106, was the subject of a study against YFV, undertaken by *Julander et al*. *en vitro*, both T-705 and T-1106 were tested in Vero cells, but

results showed only weak activity [13]. Interestingly, when tested in a rodent model, T-1106 showed significant efficacy, reducing viral load and vastly improving mortality rates against YFV [16].

With the various studies done on both T-1106 and T-705 and their RdRp inhibition properties that have been met with impressive *in vivo* results in *Flaviviridae,* as well as other RNA virus models, an efficacy comparison of T-705 and T-1106 treatment for DNV in a rodent model is the target of this proposal. Exploration of this Rdrp-targeted DENV treatment could provide additional insight to the intricate virulence factors DENV possesses, other modes of activity of T-1106 and T-705 in an in vivo rodent model for DENV, as well as the advancement of DENV antiviral research toward the consummate goal of human treatment.

Experimental Design

My hypothesis is that T-705 and T-1106 will impair the activity of DENV RdRp by acting as GTP-competitive inhibitor as observed in other *Flaviviridae* rodent models [16]. By doing an efficacy comparison, we may be able to show which treatment would be most worthy of further anti-DENV research development.

DENV receptive BALB/c mice (anticipated weight near 20g) will be obtained. After a 24-hour quarantine and 1-week acclimation period, a first group of mice will be DENV challenged by injection to determine both viral lethal dose and appropriate virus dilution for the experiment. Once these initial parameters have been defined, mice will be divided into 3 main groups: T-705 group (TG5), T-1106 group (TG6) and a placebo group (TPG) that will serve as a control group. Experimental group 1 (EG1) will consist of selected mice

from the TG5 and TG6 group that will be administered their respective antiviral 4 hours before DENV challenge, then daily thereafter for 7 days. Among this group there will be a division of oral administration (EG1O) and peritoneal inoculation (EG1P), each of which will have further group divisions based on the following dosages: 50/mg/kg/day, 200 mg/kg/day, and 400 mg/kg/day [13].

Two additional experimental groups will be established (EG2, EG3), also consisting of selected mice from TG5 and TG6. EG2 will follow the model of EG1 except that mice will first undergo a DENV challenge, foregoing respective antiviral administration until day 2. EG3 will similarly follow the model of EG1, but forego antiviral administration until day 4. The TPG group will be divided into positive and negative control groups. TPG1, the positive control group, will follow the drug administration models of EG1, EG2, and EG3, both oral and peritoneal, but will be not be DENV challenged. TPG2, the negative control group, will be DENV challenged but administered placebo antivirals in parallel with administration protocol of groups EG1, EG2, and EG3, respectively **(Figure 1)**.

Viral loads and platelet counts will be tested at 6 hour intervals to track antiviral response. Selected mice from each group and corresponding subgroup will be euthanized at various intervals to explore T cell, various other immune cell counts as well as histopathology images.

An attempt will be made to explore DHF/DSS development and the effects of T-705 and T-1106 treatment 4 weeks post-recovery of surviving mice by subsequent DENV challenge with divergent serotype. Experimental groups will be set up as before unless the initial experimental results require experimental design adjustments.

This experimental design consisting of different variable alteration groups and sub-groups will allow a comprehensive comparative analysis of DENV progression and antiviral effectiveness through data analysis of morbidity, mortality, and recovery rates as well as viral loads, cell, and platelet counts.

Anticipated Results and Interpretation

While DENV virus shares many similarities with WNV and YVF, it is possible that these pyrazine carboxamide will not show the same level of effectiveness for RdRp inhibition or general viral load reduction as seen in other *Flaviviridae* rodent models. Host enzyme reduction of pyrazine carboxamide derivatives into metabolite ribfuranosyltriphosphate is thought to be the mechanism of action that inhibits viral Rdrp, yet is not shown to disturb host DNA & RNA synthesis in WNV and YVF models. Virulence factors uniquely possessed by DENV, but not yet realized could interfere with this mechanism. Despite this possibility, the need for DENV antiviral development, combined with the promising results seen for T-705 and T-1106 in previous rodent models, makes this proposal a necessary avenue for exploration [21]. It is anticipated that data analysis will indeed reveal novel anomalies not seen in other viral models due to the unique pathogenicity and virulence factors of DENV. It is further anticipated that remarkable evolutionary conservation of viral RdRps, especially within respective RNA polarity groups, will allow DENV RdRp disruption similarly observed in WNV and YFV pyrazine carboximide studies [11]. The prospect of this novel data and potential contribution to the understanding of DENV *in vivo* will be an invaluable contribution to understanding pathogen activity and host interaction *in vivo*.

Conclusion

Today, 2.5 Billion people, or 40% of the world's population, live in Dengue epidemic regions. With the accelerated worldwide dissemination and high-adaptability of *A. aegypti* and *A. albopictus*, combined with socioeconomic restrictions on mosquito prevention and control, alternative solutions to DENV response need to be explored [4, 6, 21]. While a Dengue vaccine would be ideal, the challenge posed by varying serotypes and their cooperative pathogenic enhancement is prohibitive and post-infection antiviral treatment would still serve as a very useful tool in battling Dengue outbreaks. A key innovation on the road to human antiviral and anti-DENV treatment is an effective mouse model that allows for disease attribute exploration as well as testing of promising therapeutic solutions [21]. It is the aim of this proposal to serve such functions and contribute to the body of knowledge of both the virological and overall scientific communities. It is through models such as this, that the prospect for effective human antiviral therapies come one step closer to realization and the outlook for DENV becomes optimistic.

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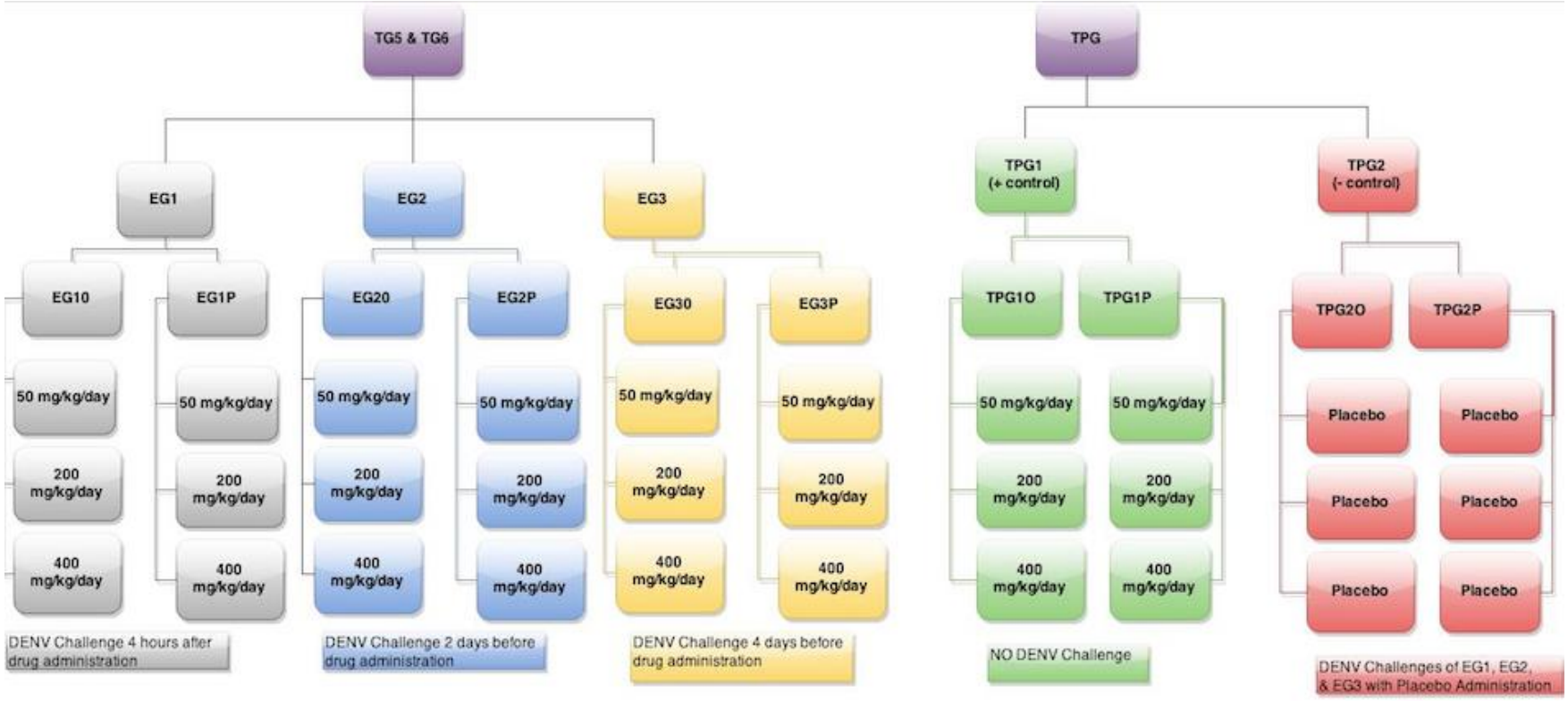

Figure 1. Experimental design mice groupings schematic